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ORIGINAL ARTICLE

Hormonal regulatory role of eyestalk factors on growth of heart in mud crab, *Scylla serrata*

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Abstract The present study was attempted to know the growth regulation of eyestalk factors on the growth of heart in *Scylla serrata* using eyestalk extractions and bilateral eyestalk ablations. The bilateral eyestalk ablation led to the maximum growth indices of the heart ((H) indices) to 0.162 and 0.158 in ablated male and female, respectively, in comparison to 0.153 and 0.167 in the control male and female and 0.147 and 0.157 in injected male and female, respectively. The data have shown that the heart of male crabs grows faster than female crabs. The study has also shown that bilateral eyestalk ablation resulted in a significant increase in the heart indices in males and has least effect on the growth of the female heart. The results presented strongly support a potential role of the eyestalk factors and molting hormone regulating the growth of the heart in *S. serrata*.

1. Introduction

The eyestalks of crustaceans contain neurosecretory cells that involve in the regulation of molting (Meade and Watts, 2001). Molting in crustaceans was thought to be regulated by two hormones; (1) molt-inhibiting hormone and (2) molting hormone. It is believed that the molt inhibiting hormone is produced in the eyestalk and stored in the sinus gland whereas the molting hormone is produced in the Y-organ. By eyestalk ablation, the molt inhibiting hormone is excluded which allows the molting hormone to act. Thus the removal of eyestalks causes an increase in ecdysteroid secretion from the Y-organ, which induces precocious molting (Nakatsuji and Sonobe, 2004; Venkitraman et al., 2004). Among all known stimuli to molting, eyestalk ablation is the most effective one in terms of the time needed to response (Chen et al., 1995), which directly affects the endocrine system of crayfish (Fingerman, 1987; Huner, 1990). Many biologists worked on the endocrine control of molting and growth in decapod crustaceans (Smith, 1940; Scudamore, 1947; Bliss, 1966; Huner and Avault, 1977; Ponnuchamy et al., 1981; Huner and Lindqvist, 1984; Chen et al., 1995; Venkitraman et al., 2004). In recent years, a few preliminary investigations have been accomplished on *Procambarus clarkii* (Chen et al., 1995), *Procambarus zonangulus* (Hobbs and Hobbs, 1990) and *Cherax quadricarinatus* (Sagi et al., 1997). Removal of eyestalk leads to weight increase

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and osmolality decrease of hemolymph (Meade and Watts, 2001; Venkitraman et al., 2004) and unilateral eyestalk ablation has been employed to induce ovarian maturation and spawning with varying success in many crustacean species (Zaib and Nisa, 2001). It can also be used to shorten the molt interval and to stimulate gonad development in decapoda (Venkitraman et al., 2004). Although the bilateral eyestalk ablation shortens the molt interval, it can also cause additional mortality (Bittner and Kopanda, 1973; Huner and Lindqvist, 1984), primarily because ablation simultaneously removes the four ganglia in each eyestalk, a considerable portion of the central nervous system (Chen et al., 1995). Therefore, ablation induced hormonal imbalance or stress, which often results in high mortality (Chen et al., 1993).

Since, no work has been done in hormonal regulatory role of eyestalk factors on growth of heart in crustaceans. So in this paper, an attempt has been made to study the growth changes in heart due to eyestalk factors and ablation in *Scylla serrata*.

2. Material and methods

2.1. Collection of crabs

Mangrove crabs *S. serrata* were collected from Parangipettai mangrove environment (Lat 11°29′N; Long. 79°). The experimental duration was 27 days. Immediately after collection, the crabs were brought to the laboratory and acclimatized to its conditions. The animals were sorted according to the size and sex and stocked separately. Healthy crabs ($n = 18$) were selected and stocked in individual troughs. The animals were divided into three groups, control, ablated and injected animals to which eyestalk extracts were injected.

2.2. Weight measurements

Weights of the heart were taken using an electronic balance after blotting the hearts on a filter paper. Wet weights of the heart in each experimental group were measured and recorded at 27 days period.

2.3. Feeding

The crabs were fed with fresh fish twice per day. For feeding, food, approximately equivalent to 20% of the body weight of the animal was used. The unused food was removed after 6–8 h. The PVC troughs were cleaned daily.

2.4. Crab saline

Saline was prepared according to Morris and McMahon (1989). The composition of the saline was 468 Mm NaCl, 11 mM $CaCl_2{\cdot}2H_2O$, 9 Mm KCl, 13 $MgSO_4{\cdot}7H_2O$, 9 Mm $MgCl_2{\cdot}6H_2O$ and 2 mM $NaHCO_3$ and pH adjusted to 7.6. It was stored at 4 °C.

2.5. Heart collection

Crabs selected for the extraction of hearts were first euthanized by submergence into salt water containing ice. The hearts were then removed from the crabs by cutting along the dorsal surface just below the cuticle to ensure no perforation of tissue occurred. The incision was then carefully opened and the hearts were removed. After extraction, the hearts were placed in labeled petri dishes.

2.6. Eyestalk ablation

Different procedures such as constriction of eyestalk with catgut, cauterization, enucleation and surgical removal of eyestalk are used for eyestalk extirpation of the X-organ sinus gland complex, which are all successful in unilateral eyestalk ablation (Venkitraman et al., 2004). In the present study, eyestalks were removed using sterile surgical blades, which reduced mortality to the minimum level. The crabs used were in the intermolt stage. During ablation, in order to reduce heartbeat, loss of hemolymph and to control bleeding specimens held in pre-cooled water and vitamin A–D ointment was applied on the sore.

2.7. Extract preparation

Eyestalks ($n = 30$) isolated from crabs were homogenized with 0.3 ml crab saline, and centrifuged at 3,000 rpm for 10 min at 2 °C. The supernatant was collected in a pre-chilled microcentrifuge tube and the homogenate re-extracted as before. The final supernatant was aliquoted into cold microcentrifuge tubes and stored at −20 °C until required.

2.8. Injection

To observe the physiological effect of injection, a single dose of 1.5 equiv sinus gland extract of the isolated supernatant was injected into each crab of 3rd group. Injections were made through the arthrodial membrane at the base of the coxa of the third pair of walking legs.

2.9. Heart indices

Heart indices were determined using the standard formula:

$$\text{Heart index} = \frac{\text{Wet weight of the heart}}{\text{Wet weight of the crab}} \times 100$$

2.10. Data analysis

Data were treated statistically by one way analysis of variance (ANOVA) to test the significance.

3. Results and discussion

Survival of crabs was 83.3%. Ablation of eyestalks significantly increased the food consumption rate of crabs and was found to have a significant effect on the growth of the heart (Table 1) of these animals. To investigate the (H) indices of experimental crabs after injection and ablation, a comparison between injected, control and ablated animals was made. The results showed that the injected and control crabs had low (H) indices than the ablated animals throughout the experimental period. The wet weight of crabs, hearts and (H) indices are shown in Table 1. Among the three groups used, the maximum growth of heart was noticed in ablated male crabs. One way

Table 1 Effect of ablation and eyestalk extraction on growth of the heart in *Scylla serrata*.

	Groups	Weight of crab	Weight of heart	(H) indices	
Control	Male	35.7 ± 2.43	0.055 ± 0.004	0.153 ± 0.004	$P < 0.05$
	Female	37.15 ± 0.49	0.058 ± 0.0007	0.157 ± 0	
Ablated	Male	34.5 ± 1.90	0.056 ± 0.003	0.162 ± 0.001	
	Female	36.85 ± 0.07	0.058 ± 0.0007	0.158 ± 0.0001	
ESE injected	Male	35.03 ± 2.19	0.054 ± 0.002	0.147 ± 0.003	
	Female	36.5 ± 0.07	0.057 ± 0.0007	0.157 ± 0.002	

Note: ESE = eyestalk extraction.

analysis of variance done showed that growth indices of the heart among these three groups differ significantly ($P < 0.05$), showing that eyestalk factors have more growth regulatory effect on the heart of male crab, *S. serrata*.

Sanjeevraj et al. (1997) in a study showed that molting and growth rates in eyestalk-ablated prawns were higher than that of nonablated ones. In an experiment conducted on destalked *M. rosenbergii*, Okumura and Katsumi (2001) reported a rapid increase of ecdysteroid and a significant shorter molting interval in comparison with intact prawns. In other studies conducted on three species including, *Astacus astacus* (Huner and Lindqvist, 1984; Gydemon and Westin, 1988), *P. clarkii* (Chen et al., 1995) and *Metapenaeus dobsoni* (Venkitraman et al., 2004), a similar significant relationship was observed between the eyestalk ablation and the growth rate. Thus the finding of the present study corroborates the above works in a sense that eyestalk factors and hormones of the Y-organ are responsible for the growth of heart in mud crab. In the present study, low mortality was observed. As shown in the present study, eye stalk factors are a key controlling factor for the growth of heart of mud crab, *S. serrata*. In general, the level of ecdysteroids in hemolymph is low throughout intermolt (stages C1–4), rises during premolt (stages D0–4), typically peaking in D2–3, then falls prior to molting, resulting in a low level of ecdysteroids during ecdysis (stage E) and postmolt. The synthesis of ecdysteroids by Y-organs is negatively regulated (inhibited) by a peptide neurohormone, molt-inhibiting hormone (MIH). MIH is produced in a cluster of eyestalk neurosecretory cell soma (the X-organ) and released from their associated axon terminals in the neurohemal sinus gland (Bruce and Change, 1984). Thus, ablation of the eyestalks lead to enhanced ecdysteroid secretion by Y-organs, an increase in the ecdysteroid titer, and precocious molting, while injection of eyestalk extract or synthetic MIH into eyestalk-ablated animals lowers the ecdysteroid titer and delays molting and growth.

The removal of eye stalks caused decreased secretion of the molting inhibiting hormone and increased the secretion of ecdysone, which may be responsible for growth of heart in *S. serrata*. Ecdysone is believed to be derived from a precursor sterol (cholesterol). This ecdysone secreted by Y-organ is responsible for molting in crustaceans when the extraction of molting inhibiting hormone was injected into the test animals; it inhibited the secretion of ecdysone by the Y-organ and lengthened their molting process and decreased their growth.

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